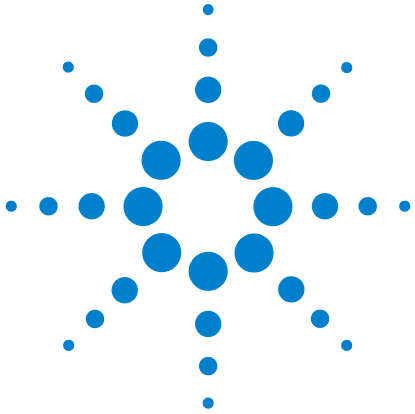


# Agilent 240 Ion Trap GC/MS

## Hybrid Ionization Users Guide

Agilent Technologies

# Notices



## Manual Part Number

G3931-90004

## Edition

First edition, May 2011

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Boulevard
Santa Clara, CA 95051 USA

## Safety Notices

**CAUTION**

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

**WARNING**

**A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.**

# Contents


## 1 Sample Analysis

Overview 5
- View reagent ions 6

Sample Introduction 7
- Ionization of the reagent gas 7
- Transferring and trapping reagent ions 8
- Sample ionization 8
- Ion storage 9
- Selectivity considerations 11

Setting Up CI Reagents 13
- Installing methane CI 13
- Adjusting CI gas flow 13

Acquiring Data 14
- Activating a method 15
- Injecting a single sample 16
- Injecting from a Sample List 17
- Monitoring run status 18

## 2 Starting the Instrument

Initial Pump Down 19
- Check the vacuum status 20
- Diagnostic tests 21
- Set system temperatures 22
- Startup and shutdown 23
- Diagnostic checks 25
- Adjusting and tuning 25
- Auto Tune 27

## 3 Creating Methods

Scan Functions 31

Using the Wizard for New Method 33
- Edit the method segments 37
- Viewing method in manual control 44

## 4 Mode Conversion

- Internal to Hybrid 49
- External to Hybrid 49
- Effects of hardware changes 50

# 1 Sample Analysis

## Overview

The Hybrid configuration is one of the three operational configurations of the Agilent 240 Ion Trap GC/MS system. In Hybrid configuration, reagent ions are generated in the external source through Electron Ionization (EI) of a reagent gas. These reagent ions are then drawn into the ion trap to react with analytes eluting from the GC column. These reactions create analyte ions, which are held in the ion trap.

The advantages of this technique include avoiding ion-molecule reactions with the neutral reagent and avoiding losses of negative ions that occur when they move from the external source to the trap.

Hybrid CI is a softer ionization technique than EI. That is, Hybrid CI imparts less energy to the sample molecules than does EI. Thus, the ionized sample molecule undergoes less fragmentation, and an ion indicative of the analyte molecular weight is more likely to be observed. In addition to molecular weight confirmation, Hybrid CI mass spectra often provide significant structural information that may not be available from EI mass spectra.

The Hybrid mode requires the external ionization option, chemical ionization option, and a security chip but does not involve any unique hardware. In hybrid mode, the external source must be in place and the transfer line must be positioned with the sample directly entering the ion trap. In common with the other configurations, it is possible to perform ion preparation techniques including Selected Ion Storage (SIS), and with optional software and equipment, Tandem Mass Spectrometry, Automated Methods Development (AMD), MS/MS, $MS^n$, and Multiple Reaction Monitoring (MRM). See the 240 Ion Trap GC/MS Software Operation help for more information.

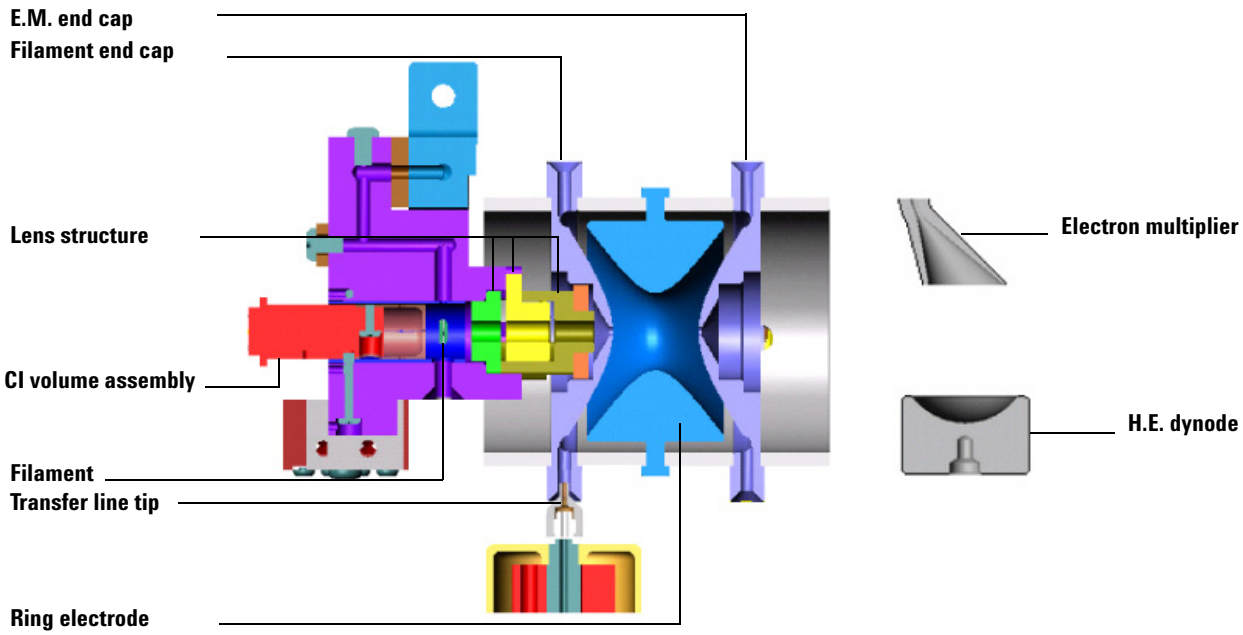
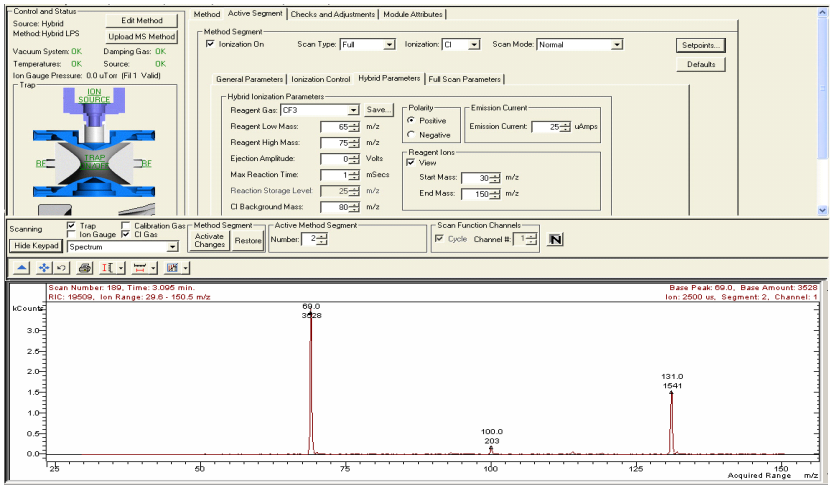



**Figure 1** Schematic diagram of the hybrid ionization configuration

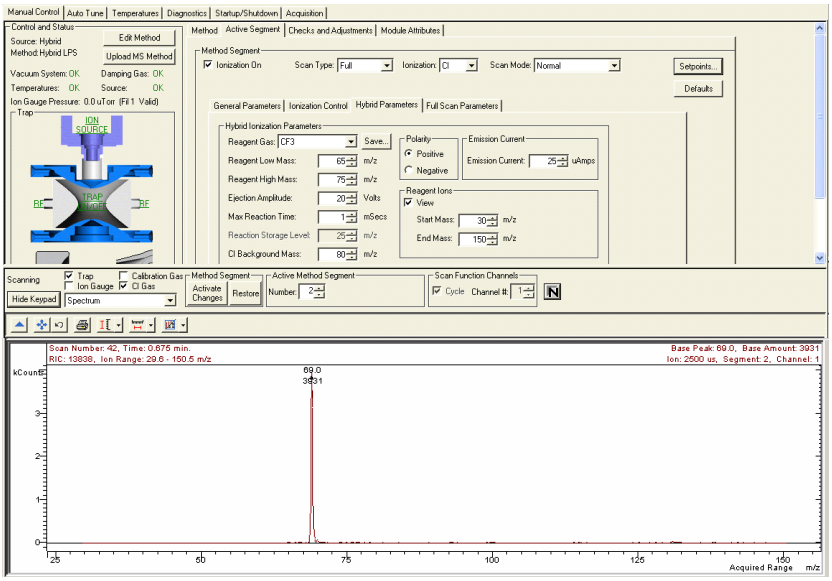
## View reagent ions

Use View Reagent Ions to tune the isolation of individual reagent ions (see the following figures). In the first figure, view all ions as set in the Reagent Ion Start and End Masses.

Because no Ejection Amplitude is specified, all the ions in the range can be viewed:



In the next figure, the Ejection Amplitude is set at 20 V, thereby eliminating the ion at 100 m/z and 131 m/z.




# Sample Introduction

Compounds are introduced through a GC column via a transfer line to the internal ion source.

## Ionization of the reagent gas

**Positive Chemical Ionization (PCI)** A reagent gas is introduced in the ion trap and EI is performed on that gas to form reagent ions. The reagent ions then undergo ion-molecule reactions with the sample molecules to create ions of the sample molecules and their fragments.

Reagent ion formation can be complex. For example, when methane is used as the reagent gas, reagent gas ions are formed as follows:

First, methane is ionized to form two primary ions:

$$CH_4 + e^- \rightarrow (CH_4^{\bullet})^+ + 2e^-$$
$$CH_4 + e^- \rightarrow CH_3^+ + e^- + H^-$$

These primary ions then react very rapidly to form predominantly the secondary ions, $CH_5^+$ and $C_2CH_5^+$ :

$(CH_4^{\bullet})^+ + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$

$CH_3^+ + CH_4 \rightarrow C_2CH_5^+ + H_2$

Stable negative ions are formed in the external ion source under electron ionization.

For example, methanol CI reagent forms a stable negative ion of m/z 31:

$CH_3OH + e^- \rightarrow (CH_3OH)^{-\bullet}$

$(CH_3OH)^{+\bullet} + CH_3OH \rightarrow CH_3O^{\bullet} + CH_3O^- + H_2$

## Transferring and trapping reagent ions

The reagent ions are transferred to the ion trap by applying voltages of the opposite polarity to the three lenses between the ion source and the ion trap. Lens voltages are negative for Hybrid PCI and positive for Hybrid NCI. The voltages on the lenses are tuned in Auto Tune to optimize focusing the ions toward the ion trap. The Trap DC offset voltage applied to the ion trap creates a potential well to trap all ions above a mass determined by the RF Storage Level. The default RF storage level is 35u, so only ions above this m/z are stored in the ion trap. Therefore, the CI reagent ions at m/z 17 and 29 are not stored and only reagent ions above 35u react with analyte molecules. This ability to select the reagent ion can add to the selectivity of the Hybrid mode of operation.

## Sample ionization

In the second step, sample molecules eluting from the GC column are ionized in the mass spectrometer via either Positive Chemical Ionization or Negative Chemical Ionization.

**Positive Chemical Ionization**: Ion-molecule reactions between the positively charged reagent ions and the GC analytes.

There are four principal reactions between reagent gas ions and sample molecules. They are:

(A) Proton transfer: $(RH)^+ + M \rightarrow (MH)^+ + R$

(B) Hydride abstraction: $R^+ + M \rightarrow (M - H)^+ + RH$

(C) Association: $R^+ + M \rightarrow (MR)^+$

(D) Charge transfer: $R^+ + M \rightarrow M^+ + R$

where $R^+$ is the secondary reagent gas ion and M is the neutral sample molecule.

For Hybrid CI using methane, proton transfer (A) is a major reaction, and association (C) is the next most often observed reaction. In both cases the resulting even-electron ions are often relatively stable, and strong (M+1) protonated molecules or (M+29) and (M+41) adduct ions are often observed even if the EI spectrum of the same component shows no molecular ion. Methane is recommended as the most useful PCI reagent gas in the Hybrid configuration.

**Negative Chemical Ionization**: Negatively charged low-energy electrons attach to GC sample molecules with high electron affinities.

Methane serves a different function in negative chemical ionization than it does in PCI. Besides ionizing methane in the source, electrons striking methane transfer much of their energy to the methane molecules and ions during the process. When the methane pressure in the source is high enough so that there are many collisions between methane molecules and electrons, this energy transfer eventually thermalizes the electron energy to levels of less than 1 eV. When electron energy is this low, attachment to molecules with high electron affinities is possible.

## Ion storage

Following reaction between the reagent ions and the analyte, analyte ions are stored and stabilized in the ion trap cavity by an RF field applied to the ring electrode of the ion trap. During ionization, the voltage of this RF field is relatively low so that ions of the entire desired mass range are stored. An auxiliary helium gas flow to the ion trap buffers the ion motion and focuses the ions more to the center of the trap. Helium is used as the buffer gas because heavier gases would give poor mass spectral resolution. Use a flow rate of 1 mL/min.

### Ion preparation

After ions are stored in the trap, they can be manipulated. A combination of waveforms can be applied to the ion trap electrodes to isolate or remove specific ions after they are formed and stored in the ion trap.

Options like Tandem Mass Spec (MS/MS) and Selected Ion Storage (SIS) can be performed on the ions stored in the ion trap before mass analysis takes place. In MS/MS, a parent ion is

isolated and then dissociated by energetic collisions with helium buffer gas to form product ions. In SIS, resonant waveforms are applied to eject unwanted ions within the stored mass range and fill the trap only with ions in the mass range(s) of interest. Advantages associated with ion preparation methods are similar to those of other sample preparation methods, such as reduction of noise and increased selectivity.

The Hybrid configuration has SIS, MS/MS, $MS^n$, and MRM as ion preparation options. SIS is included with all instruments, while MS/MS, $MS^n$, and Multiple Reaction Monitoring (MRM) are available with the MS/MS option installed.

### Ion analysis

The stored ions are ramped by the RF voltage applied to the ring electrode to a high value. Ions, from low to high mass, are successively destabilized and ejected from the trap. Supplemental dipole and quadrupole voltages applied to the end cap electrodes improve the mass resolution of the process. After being ejected, the ions strike a conversion dynode, initiating a signal amplification process at the electron multiplier.

The ion trap has a maximum storage capacity beyond which mass resolution and spectral quality deteriorate. The number of ions created is proportional to the ionization time; more ions are produced the longer the ionization time. Automatic Gain Control (AGC) controls the ionization time to always create an optimum number of ions in the trap.

The AGC scan function consists of a prescan and up to six analytical scan segments. The number of ions detected in the prescan is used to calculate the ionization time for the analytical scan.

All ions with masses above a chosen value set by the RF Storage Level are stored in the ion trap and ions higher than the selected high mass limit are eliminated by waveforms applied to the end caps. See the 240 Ion Trap GC/MS Software Operation help for more information.

### Scanning ions to collect mass spectra

The scanning process for Hybrid chemical ionization is the same as for electron ionization. After ionization, trapping, and ion preparation steps, ions are scanned out to the conversion dynode and electron multiplier. Mass scanning is implemented by increasing the RF voltage on the ring electrode; the mass spectrum is collected in order from low to high mass over the

user-designated scan range. Ions ejected from the ion trap are attracted to the conversion dynode. In positive modes, electrons are ejected from the conversion dynode, held at –10,000 V, and repelled to the electron multiplier. In negative mode, positive ions are ejected from the dynode, held at +10,000 V, and repelled toward the electron multiplier. The signal is amplified by ~$10^5$ by the multiplier and sent through an integrator to collect an intensity for each m/z. MS data are stored as sets of ion-intensity pairs for each m/z over the acquired mass range. A complete mass spectrum is stored for each analytical scan. There are two types of mass scanning in Hybrid CI. First, is a prescan to count the number of ions formed in a short fixed ion time. After a calculation based on the prescan ion count, ions are formed for the ionization time recommended by the AGC prescan algorithm and the analytical scan is carried out.

### Library searching

There are no libraries of Hybrid PCI or NCI mass spectra included with 240 MS software; however, users can create libraries of these spectra. For more information about creating a library, see the 240 Ion Trap GC/MS Software Operation help.

## Selectivity considerations

One of the advantages traditionally noted for Hybrid chemical ionization is selectivity. In Hybrid PCI, hydrocarbons have poor response in methane CI. It may therefore be much easier to locate target compounds in a hydrocarbon-contaminated sample using methane PCI than using EI. Similarly, negative CI gives a good response only for species with a high electron affinity such as halogenated compounds; chemical background from other types of species will not even show up in the chromatogram.

Because of these selectivity considerations, it is often worth the time during method development to analyze samples using the suite of different ionization and ion preparation options available on your MS system.

### Using hybrid mode to obtain more information

For many species, there is so much unimolecular fragmentation of molecular ions that there is little or no intensity in the mass spectrum to identify the molecular mass. An examination of the NIST Mass Spectral Library confirms this statement. When one is attempting to identify unknown species, the ability to select

the reagent ion may allow highly selective reagent ion/analyte reactions to assist in identifying the analyte molecular weight and isomeric configuration.

# Setting Up CI Reagents

Although several liquid and gaseous reagents are useful in Hybrid configuration, methane appears to be the reagent of choice. Liquid reagents like methanol and acetonitrile give weak responses for most analytes in Hybrid positive chemical ionization, PCI.

## Installing methane CI

For full details on installing a CI gas, see the “Installing a CI Reagent Gas” section in the 240 Ion Trap GC/MS Hardware Operation Manual.

To install a gaseous reagent, do the following:

1 Connect the regulator of the gas cylinder to the back of the instrument through a 50 mL/min restrictor.

2 Open the methane tank and set the second stage of the regulator to 20 psi.

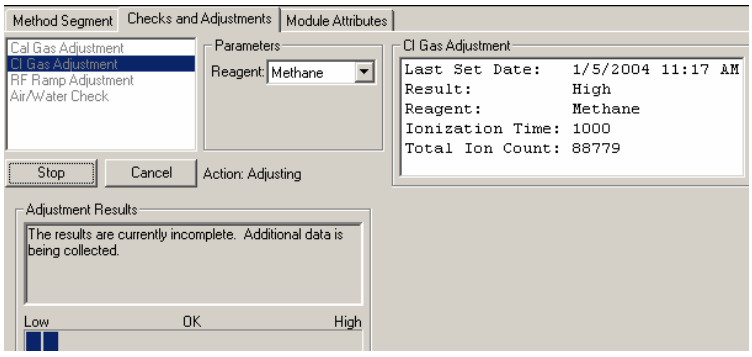
3 See the 240 Ion Trap GC/MS Hardware Operation Manual for more details.

## Adjusting CI gas flow

1 Open the Checks and Adjustments tab dialog in Manual Control.

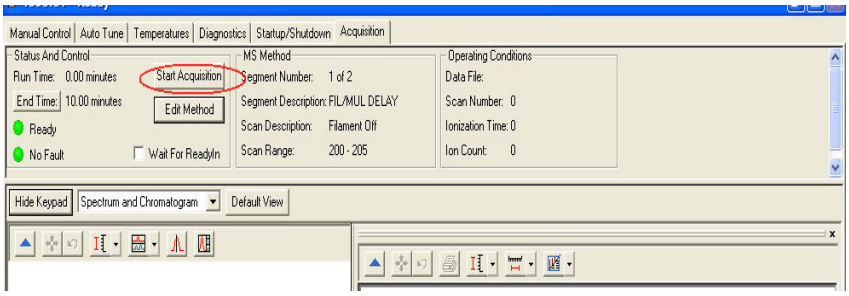
2 Click **CI Gas Adjustment** and click the **Start** button.

3 Use the CI Gas Adjust Valve inside the front door of the 240 MS. Turn the knob clockwise to increase the flow or counterclockwise to decrease the flow. The objective is to set the ion gauge pressure within the range of 70 to 100 µTorr. Adjust the gas until the adjustment result is OK.



# Acquiring Data

Click **Start Acquisition** to start your run. If you start an analysis while the instrument is in another mode, the software automatically shifts the MS module into Acquisition mode.




If the GC is not ready, you will see a Not Ready message at the top of the screen. After the GC and AutoSampler come to a ready state, the Not Ready message will change to Ready. To determine the individual ready states of the components, you can go to the top pull down menu under Windows and see the states for the 240 MS, 7890 GC, and sampler modules. After components are ready, you can start an analysis.

An analysis can be run as a single sample or as an automated sequence.

To run a single sample, do the following:

- To run in manual mode, see “Injecting a single sample" on page 16
- To run in automation mode, see “Injecting from a Sample List" on page 17“

You can run both single samples and sample lists using from QuickStart. For more information on using QuickStart, see the 240 Ion Trap GC/MS Software Operation help.

### Status and control

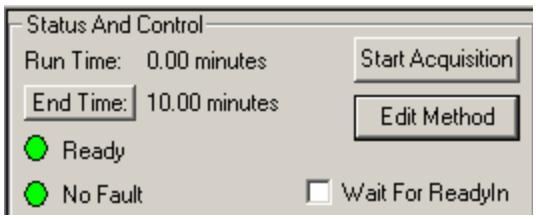
Before an acquisition starts, the Status and Control field will look like the following figure.

- The Run Time will be 0.00 minutes.
- The End Time will be the run length specified for the 240 MS module in the active method.
- The Ready and No Fault lights are green.

You can click the Start Acquisition button to override automation and start a run even before the system comes to Ready. However, the file name of a run started in this way will be named as 4000.x.sms, not the file name specified for automation runs.

Clicking the Edit Method button allows you to open the Method Builder and modify the method. You are prompted to re-activate the method after saving changes and are returned to System Control.

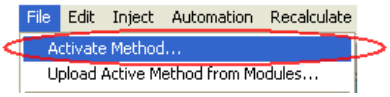
Changing the End Time for the MS module does not change the GC End Time. You must access the GC module from the Windows menu and change the GC End Time separately.




## Activating a method

1 Click the **File** menu.

2 Click **Activate Method**.

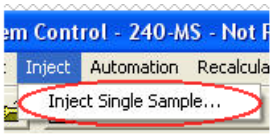



3 Select a method by either

- Clicking **Recent Files** to display the eight most recent methods.
- Clicking **Open**, after selecting a method from a folder.

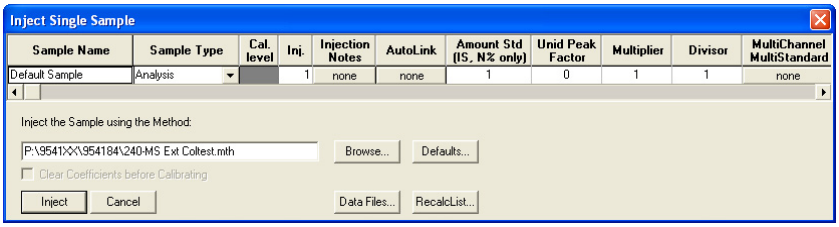
## Injecting a single sample

1 Click Inject Single Sample, from the Inject menu.




2 After the Inject Single Sample window opens do the following:

- Type a sample name.
- Enter the vial number of the sample vial if an autosampler is configured.
- Check that the injection volume and injector used are correct.
- Click **Defaults**, to change the default values for any parameter.
- Click **Data Files** to create a name that includes more information such as date and time, or to change the directory for data file storage.




3 Click **Inject** to acquire the data.

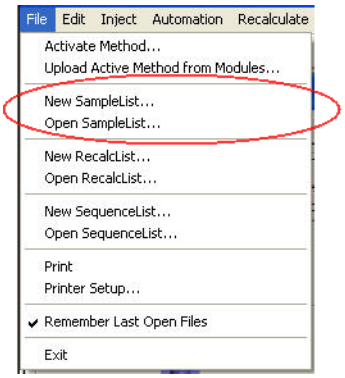
- If the MS is not in Acquisition mode, it changes to that mode automatically.
- If an AutoSampler is doing the injection, it begins after the instrument modules are Ready.
- If you are doing a manual injection, wait until the System Control title bar reads “Waiting for Injection of Sample” and there is a blinking yellow Waiting light on the right of the System Control toolbar. Then inject the sample.


## Injecting from a Sample List

You can create and edit a Sample List in the Automation File Editor or in System Control.

To edit a Sample List and inject multiple samples from System Control do the following:

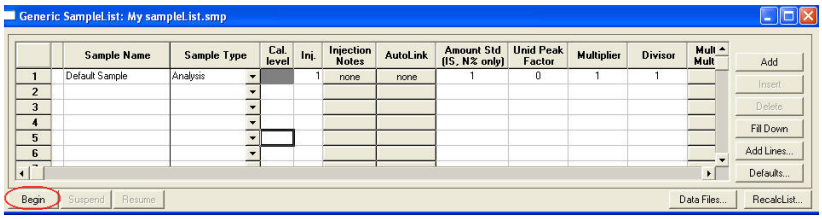
1 Click either New Sample List or Open Sample List from the File menu.




2 The Sample List window for the open Sample List opens. It contains fields that are specific to the autosampler configured. See the following figure.

- Change the size of the spreadsheet columns by dragging their border with the left button of the mouse.
- Right-click a column header for formatting options. When the table is scrolled to the right, the Sample Name column does not scroll so you can easily tell for which sample you are entering additional parameters.
- Click **Add** to add additional samples. Enter the name, sample type, and vial number for all samples.

3 Click **Begin**, in the lower left corner, to start the Sample List.

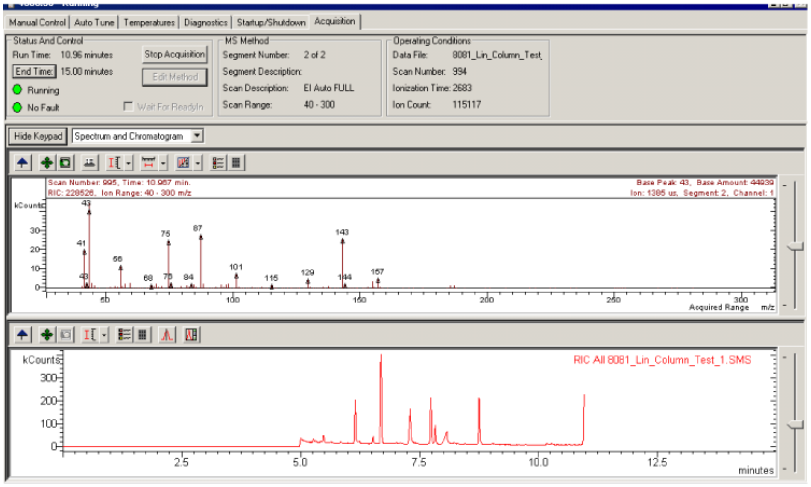



## Monitoring run status

Monitor the status of the run in the instrument window. The status and control windows and the Toolbar show the run status.

Monitor the chromatogram and spectra in System Control, or click the far right button in the Chromatogram toolbar to transfer to MS Data Review, where you can perform operations like library searching while the data file is being acquired.

For more information on data acquisition features, see the Acquiring GC/MS Data section in the 240 Ion Trap GC/MS Software Operation help.



# 2 Starting the Instrument

## Initial Pump down

Check the following:

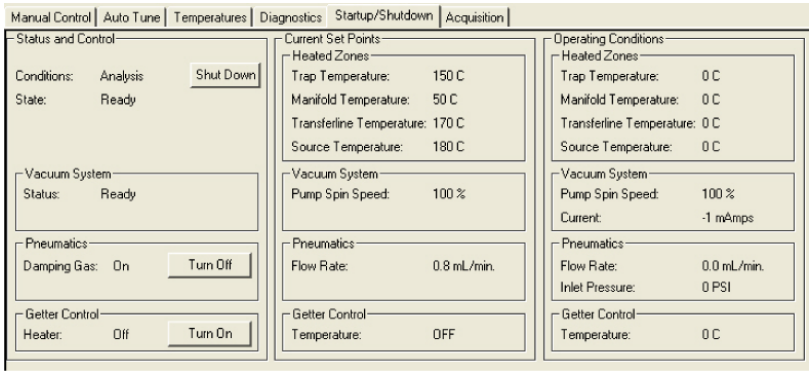
- Verify that the Vacuum connections are leak free
- Verify that the transfer line is seated in the trap
- Verify that the vent valve is closed fully clockwise
- Verify that the column is not broken

Turn on the power at the main power switch; the roughing pump should stop gurgling after about 10 to 20 seconds.

If the pump continues to gurgle, do the following:

1. Verify that the analyzer assembly is seated properly on the manifold (there should be no gaps).
2. Verify that the transfer line is seated in the trap.
3. Verify that the vent valve is sealed.

Open **System Control** and the **Startup/Shutdown** page appears.



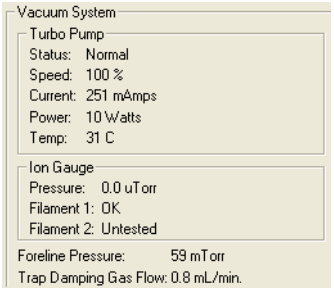
## Check the vacuum status

The vacuum readings provide much information about the MS after pump down (and during operation). Typical operating ranges for the 240 MS in Internal mode are shown in Table 1.

**Table 1** Typical operating ranges in External mode

| | |
|---|---|
| Speed | 100% |
| Current | 200 to 300 mAmps |
| Power | 9 to 13 Watts |
| Ion gauge pressure | < 20 µTorr |
| Roughing line | < 50 mTorr |

If the Pump Spin Speed does not increase steadily, there may be a leak in the system. Large leaks are indicated by a turbo speed less than 100%. Small leaks will show up by an increase in the pump current after at 100% or in the ion gauge pressure (See “Diagnostics”). Diagnose small leaks by observing changes in the ion gauge reading and pinpoint them using the leak check section in the method Service.mth. For more detail on troubleshooting leaks, see the Troubleshooting section in the 240 GC/MS Ion Trap Hardware Operation Manual.




### Start damping gas flow

The addition of damping gas may or may not improve sensitivity. Start without damping gas and then increase to 0.5 mL/min to determine if sensitivity increases.

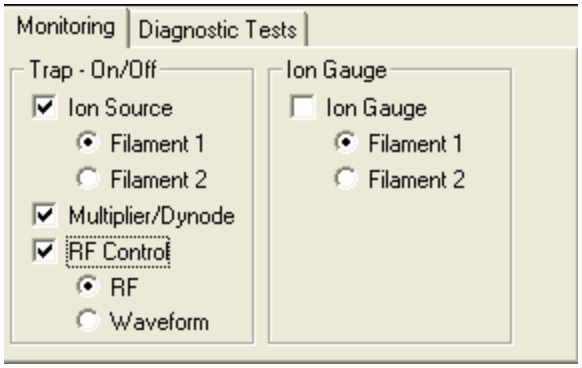
When the turbomolecular pump speed reaches 100%, turn on the Damping Gas and Getter. After the flow starts, you can check the rate in the Operating Conditions field on the right side of the dialog. The buffer flow is necessary to maintain mass

spectral resolution; helium also improves the trapping of ions entering the trap from the external source. Although trapping efficiency and therefore instrument sensitivity dependence on the helium flow rate is compound dependent, a good initial choice of flow is 3 to 4 mL/min.

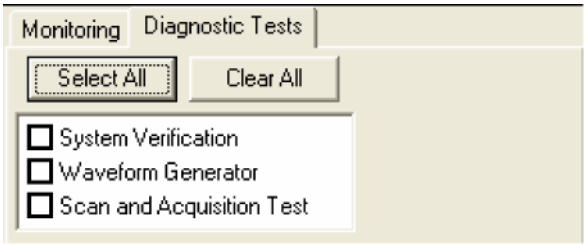
Set the helium buffer gas flow rate in the Module Attributes tab.

## Diagnostic tests

Monitor the current state of the instrument using the Monitoring tab. Monitor the vacuum system, the electron multiplier, the waveform system, temperatures, and the ion source.




Perform hardware checks on the 240 MS using the Diagnostics tab. For more details on the diagnostic tests, see the Diagnostics section in the 240 GC/MS Ion Trap Software Operation Manual. For more details on the diagnostic tests, see the Diagnostics section in the 240 GC/MS Ion Trap Software Operation Manual.



## Set system temperatures

### Analysis temperatures

Ion trap temperature is important for analyses performed in the Hybrid Configuration because it must be high enough to prevent condensation of analyte as it elutes from the GC column into the ion trap.

Changing the source temperature takes only a few minutes. However, there may be subtle effects on lens tuning and mass calibration. Perform mass calibration and trap function calibration shortly after the desired source temperature is reached and then again several hours later or the start of the next day.

Set the transfer line temperature so that there is no cold spot between the GC column oven and the MS. A transfer line temperature that is 20 °C below the maximum column temperature of the active method should be adequate.

The default manifold temperature, typically 50 °C, reduces the effects room temperature variation may have on the system.

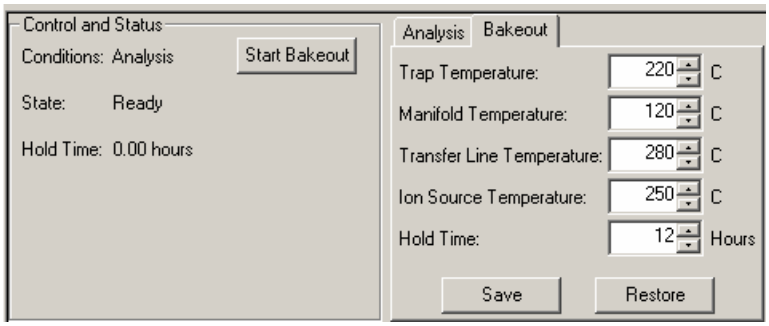
### System bakeout

To remove water adsorbed on the manifold while the 240 MS was vented, perform a Bakeout from the Temperatures tab in System Control.

Bakeout can also remove chemical background from the MS after running heavy matrix samples such as environmental or biological extracts.

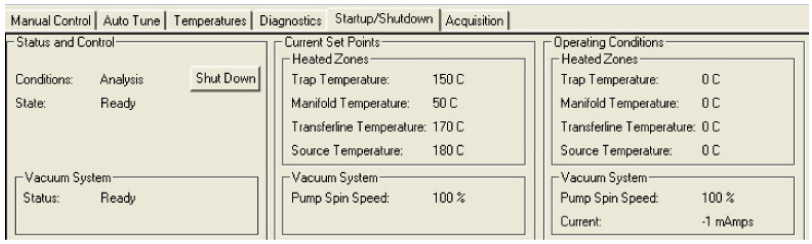
Typical bakeout settings are shown in the next figure. When Bakeout begins, the temperatures are raised to those set in the Bakeout tab dialog. The Hold Time in the Control and Status field decreases until bakeout is complete. System temperatures returned to those set in the Analysis tab. Wait at least two hours after bakeout is completed before attempting to AutoTune or run the 240 MS, allowing all temperature zones to equilibrate thoroughly.

The transfer line temperature should not exceed the maximum isothermal temperature of the column.




## Startup and shutdown

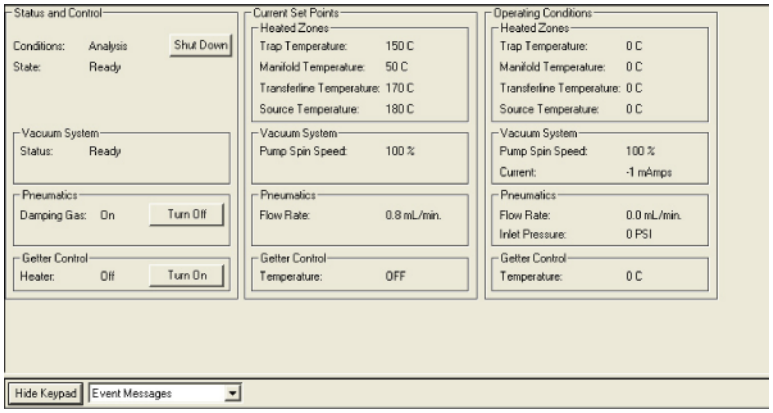
Use **Startup/Shutdown** to start up or shut down the system in a safe and orderly fashion.




### Starting the system

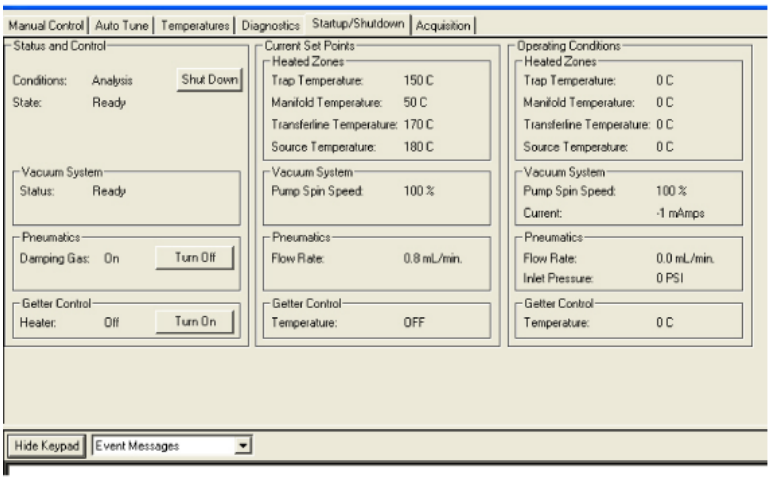
When the system first comes on, System Control operates in Startup/Shutdown mode. During system startup, you can observe the increase in turbo pump speed in the **Operating Conditions** section. The software is locked in the Startup/Shutdown mode until the speed reaches 100%. You can also see the temperature readings for heated zones rise in the **Operating Conditions** section.

Failure to reach 100% pump speed in a reasonable time indicates a vacuum leak and corrective action should be taken. For details, see the appropriate Troubleshooting section in the 240 GC/MS Ion Trap Hardware Operation Manual.




## Shutting down the system

To shut down the 240 MS, click the **Shut Down** button in the upper left corner of the screen. The heaters are turned off and the speed of the turbo pump gradually reduced to 35% of full speed. In the following figure, **Shut Down** was clicked. Note that the turbo pump speed decreases as the temperature decreases.



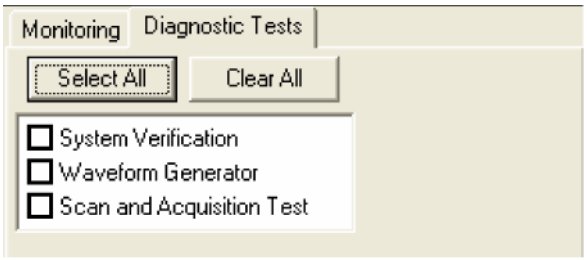

To restart the system after a shutdown, click **Start Up** on the left side of the screen. This restarts the pumps and turn on the heaters.

After all temperature zones are below 80 °C, turn OFF the main power using the switch at the rear of system. Manually vent the system for at least 5 minutes using the valve on the front panel.

Retract the transfer line before lifting the analyzer assembly from the vacuum manifold. Failure to retract the transfer line can cause damage to the transfer line tip and to the trap assembly.

## Diagnostic checks

After the turbomolecular pump reaches 100% speed, you can perform normal operations. Check for instrument problems by running all of the routines in the Diagnostic Tests tab dialog of the Diagnostics mode. Click the **Select All** button and then click the **Start Diagnostic** button in the Control and Status area to the left. If a test fails, see the relevant "Troubleshooting" section in the 240 GC/MS Hardware Operation Manual.




## Adjusting and tuning

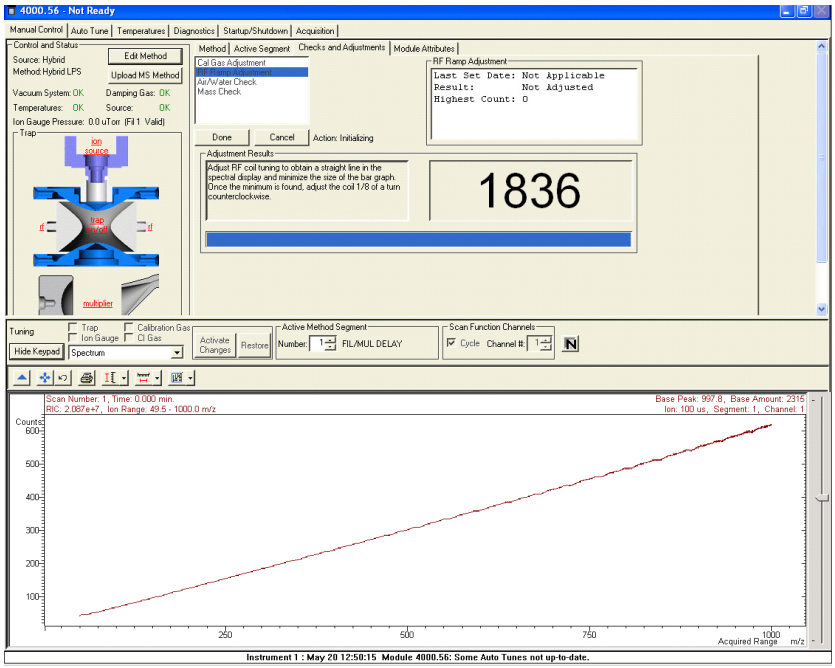
### RF tune

Adjust the RF tuning in the Checks and Adjustments tab dialog of Manual Control after performing any of the following:

- Performing MS maintenance.
- Changing the analyzer assembly.
- Changing the MS configuration.

### RF ramp adjustment

1. Click RF Ramp Adjustment in the Checks and Adjustments tab in Manual Control.
2. Click **Start**.
3. Use a flathead screwdriver to turn the RF Adjustment screw, inside the front door of the 240 MS, either clockwise or counterclockwise until the tuning display shows a straight line and the intensity is at a minimum. The Status Bar in the Adjustment Results field should be just below OK.

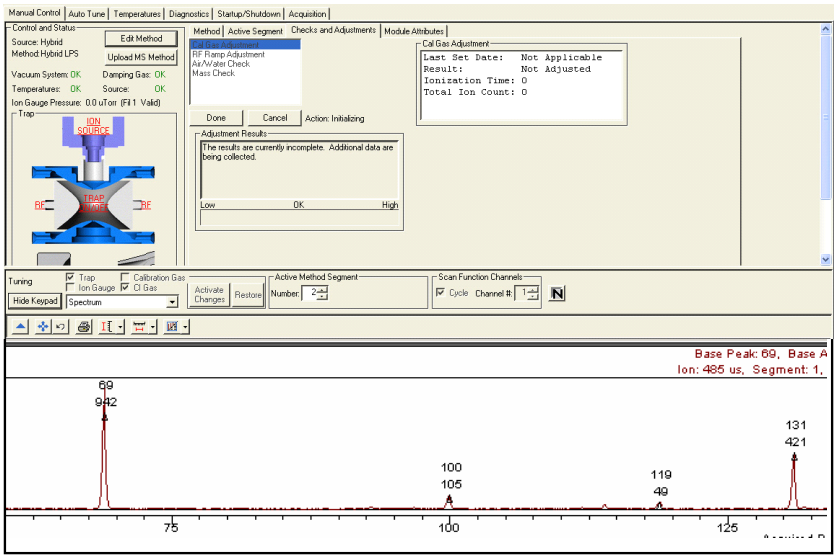



## Calibration gas adjustment

Check the flow of perfluorotributylamine (PFTBA or FC-43) calibration gas before performing Auto Tune procedures.

To adjust the calibration gas, do the following:

1. Click Cal Gas Adjustment in the Checks and Adjustments tab in Manual Control.
2. Turn the Cal Gas valve inside the front door of the 240 MS either clockwise to decrease the flow or counterclockwise to increase the flow. Adjust the flow so that the status bar in the Adjustment Results field reads OK.




### CI gas adjustment

Before acquiring data in Hybrid chemical ionization (CI) mode, adjust the CI reagent gas pressure. Details on how to set up methane CI gas are found in the section "Setting Up CI Reagents" on page 13.

### Air/water check

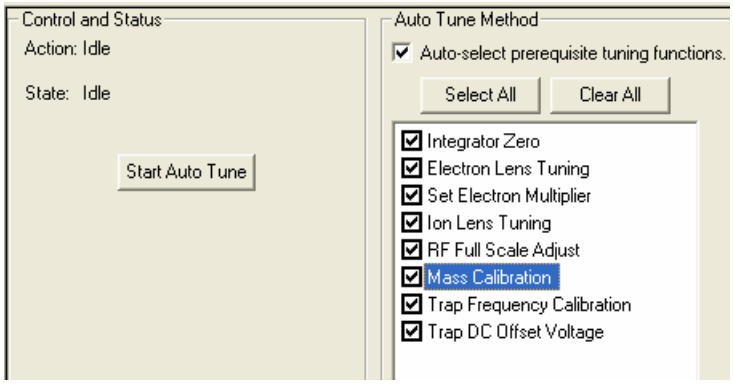
Too high a pressure of air or water in the system because of an air leak or a need to bake out the system results in poor performance. This routine provides information on the levels of air and water.

The Air/Water Check uses the electron multiplier voltage with the gain at $10^5$ and not the manual setting. If the electron multiplier is replaced, auto tune the Electron Multiplier *before* performing the Air/Water Check.

## Auto Tune

Depending on the configuration and settings, different Auto Tune routines are available. Perform auto tune when the instrument is first set up and whenever significant maintenance operations are performed. Also, perform Mass Calibration and Trap Frequency Calibration whenever the temperature or RF adjustment is changed.

Auto Tune works the same way in either EI or Hybrid CI modes; you do not need to run a different automatic setup, tuning, and calibration program for Hybrid CI.




### Integrator zero

Integrator Zero obtains the average value of the signal level coming from the integrator circuitry when the filament is off. When the filament is off, the major source of signal coming from this circuitry is electronic noise. The integrator zero is adjusted so that electronic noise does not create an artificial ion and ions from the trap striking the multiplier create a measurable signal.

### Set electron multiplier

Set Electron Multiplier determines two settings, the multiplier voltage needed to achieve a multiplier gain of approximately $10^5$, and the Electron Multiplier voltage boost for optimum peak intensity and resolution.

### Electron lens tuning

Electron lens tuning involves measuring the transient behavior of the emission current immediately after the lenses have been switched on or off. If the lenses are unbalanced, the emission current will change in time and be proportional to the imbalance. If the balance is outside the range of 200 to 300 µA, the algorithm will search the optimal values by changing values of four variables one at a time. If it fails to find the best voltage setting for lens tuning, auto tune will generate an error message, and restore the last values in the instrument.

When the Electron Lens Tuning Box is clicked, an additional “Turn on CI gas flow during tune” option appears. For CI methods in Hybrid mode, the electron/ repeller lens must be

tuned with the CI plunger (CI volume) in place and the CI gas turned on. The user should adjust the CI gas flow in Manual Control before this tune function is done.

### Ion lens tuning

The Ion Lens system consists of three lenses (Lens 1, 2 & 3). These lenses are tuned using Cal Gas ions at m/z 131 and 414. Optimum voltages are determined based on weighted intensities of the two ions. Transmission of both low and high mass ions is monitored as a function of lens voltages in this iterative process

### RF full scale adjust

RF Full Scale Adjust sets the full scale adjust potentiometer to give the correct mass assignment for high mass ions in the calibration gas spectrum. Set the RF Full Scale adjust be doing Mass Calibration and Trap Frequency Calibration.

### Mass calibration

Mass Calibration locates and correctly assigns the masses of the PFTBA calibration gas ions at m/z 69, 131, 264, 414, 464, and 614.

Ion trap temperature changes can shift the mass calibration axis; **do not run this procedure until the ion trap temperature has stabilized for at least two hours**. There could also be subtle effects on mass assignments after ion source temperature changes.

### Trap frequency calibration

After completing mass calibration, perform Trap Frequency Calibration. This calibration determines parameters required for ion preparation methods such as MS/MS and SIS. These parameters also help to isolate the range of ions to be acquired in full scan acquisitions. The routine takes several minutes.

### Trap DC offset voltage

Adjust the trap DC offset to optimize the ion signal for m/z 414 in the calibration gas. An optimal value for this parameter assures good high mass sensitivity.

# 3 Creating Methods

## Scan Functions

In the Hybrid CI configuration, the external ion source is installed, but the transfer line directs the sample into the ion trap. CI reagent ions are generated in the external source and only selected reagent ions are stored in the ion trap. These trapped reagent ions react with sample molecules as they enter the ion trap, and form CI product ions by ion-molecule reactions. Use Hybrid CI with either positively charged or negatively charged reagent ions.

The ion trap operates in a pulsed mode. Reagent ions are created only during the ionization pulse and are consumed during the reaction period to form analyte ions. The number of analyte ions depends on the concentration of the analyte, the initial reagent ion intensity, and the reaction time.

Control of Space charging is achieved by using the results from an AGC prescan to calculate the ionization time and reaction time for the analytical scan. Because the spectral intensity is proportional to sample concentration and reaction time, linear calibration curves can be obtained.

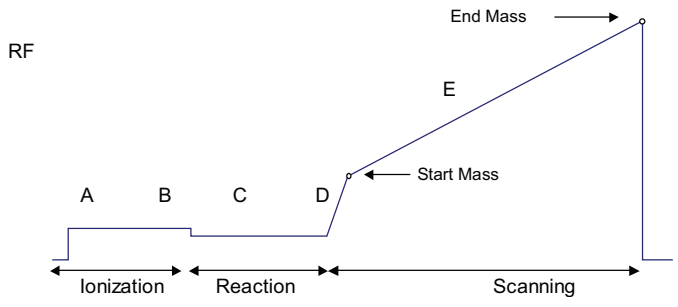



**Figure 2** Hybrid CI scan function (analytical scan only)

During the Hybrid CI analytical scan, the following steps occur:

**a** The reagent gas is ionized for the length of time determined by the prescan.

**b** The selected reagent ions are stored in the ion trap. Ejection of ions above the Selected Reagent High Mass is accomplished by applying a broadband waveform between the ionization and reaction periods.

**c** Reagent gas ions react with sample molecules to form sample ions. (The reaction time is determined by the prescan.)

**d** Reagent ions are ejected.

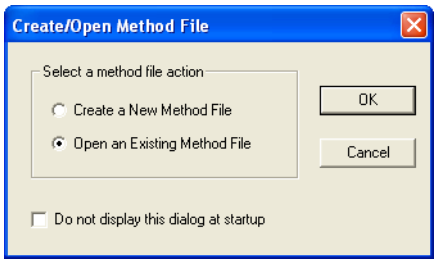
**e** The Hybrid CI mass spectrum is acquired for the sample ions.

The ionization and reaction storage RF can be set at the same level or different levels.

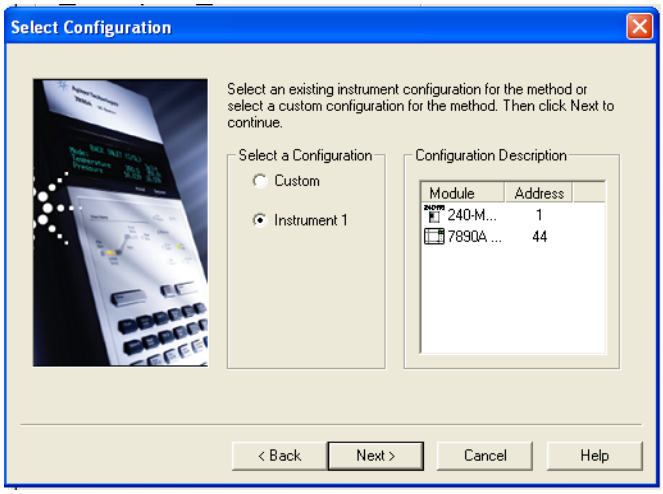
## Using the Wizard for New Method

1 Click the **Method Builder** icon on the Workstation Toolbar.

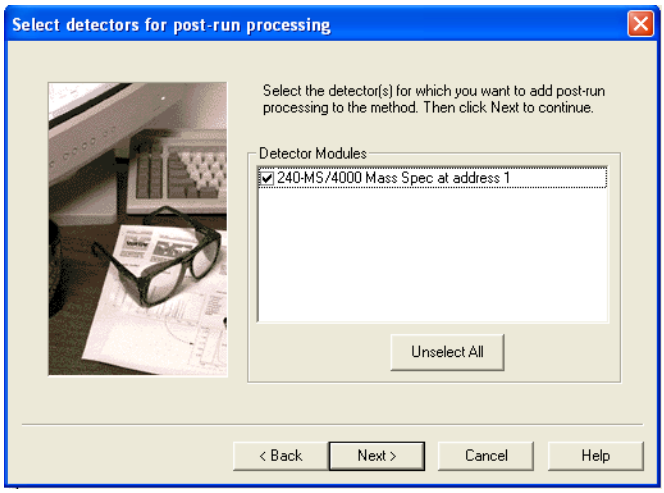
2 Click **Create a New Method File**. The Wizard guides you in building this new method. If you do not want to see this message again, check the box **Do not display this dialog at startup**.



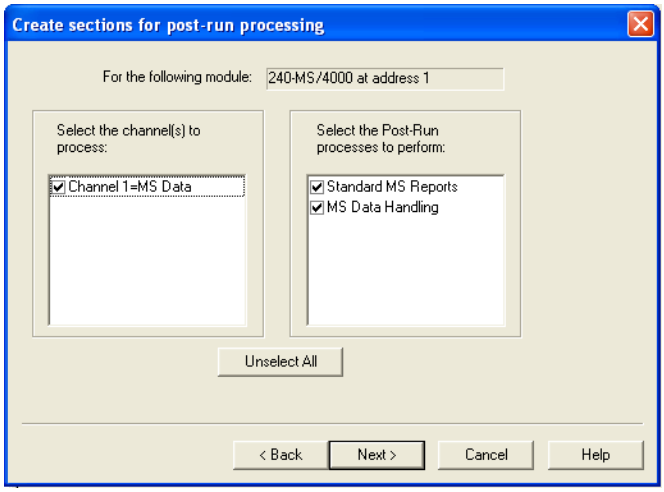

3 Select **Instrument 1** and click **Next**. Use Custom configuration to create methods on a PC remote from the instrument



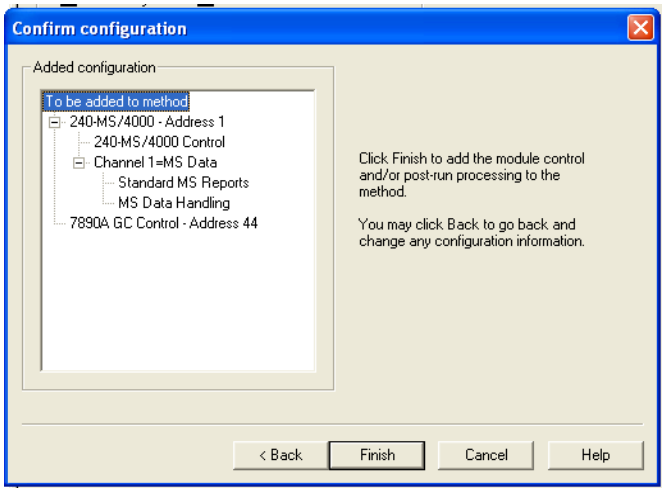
4 Select the detector(s) for post-run processing and click **Next**.




5 Select the data channels and type(s) of post-run processing for each detector and click **Next** to display the next detector if configured.




6 Click **Finish** to add the method. The wizard creates a Method containing all the sections needed to control the hardware, collect data, and do the post-run processing specified. The Method contains default values for all parameters. See the MS Workstation Software Reference Manual for information about data handling and reports.




The method contains the following sections: 7890 GC Control, 240 MS Control, Standard MS Reports, and MS Data Handling.

### Name the method

1 From the **File** menu, click **Save As**.

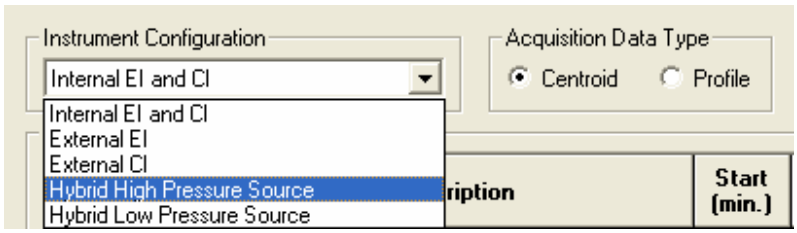
2 Type a name for the method.

3 Select the folder in which to keep the method.

4 Click **Save**.

### 240 MS instrument configuration

The configuration defines what ionization modes can be used for data acquisition. In Hybrid Mode, the configuration is Chemical Ionization (CI). The instrument Configuration is set in the upper-left corner of the MS Method Editor by selecting from the drop-down list box.



### Hybrid configuration options

Hybrid methods can be performed only in positive or negative ion CI modes (PCI or NCI) except when running Auto Tune methods requiring EI mode. For both of these methods, CI reagent ions are formed in the external ion source and drawn into the ion trap to react with compounds eluting from the GC column. Note the Hybrid HPS and LPS options in the selection menu. Hybrid HPS (High Pressure Source) is performed with the CI volume inserted into the ion source whereas the LPS (Low Pressure Source) option occurs in the EI source.

### Select the acquisition data type

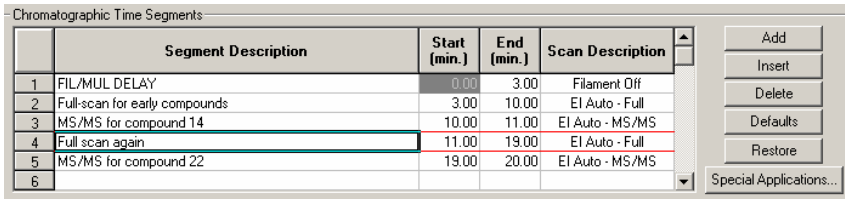
Centroid data is the default acquisition data type. Data handling, library searching, and spectral comparison can only be done using centroid data. The analog signal from the detector is sent to an analog to digital converter. The software determines the center of gravity of the digitized ion signal, the centroid. The software creates the “stick” spectrum from the digitized ion signals.

Profile data is used mainly for diagnostic purposes. Profile files are also approximately 10 times larger than centroid files, but they can be converted to centroid after acquisition.

Profile data is collected at 10 points per m/z and is displayed as peaks similar to a chromatogram. The display allows you to observe the true dispersion of the response and determine if adequate resolution has been obtained.

### Edit chromatographic time segments

Use the Chromatographic Time Segments table to time program analysis conditions to get the best results for each segment in the analysis. Up to 250 time segments can be created for runs up to 650 minutes in length. By default, there is a Filament/Multiplier Delay segment at the start of the run so that the system will not be stressed during the elution of the chromatographic solvent. Following this segment, one could just acquire the mass spectra in full-scan with a single analysis segment. However, one can tailor variables such as acquired mass range, insert MS/MS segments for individual analytes, and otherwise set up the instrument to acquire the best data for each analyte.



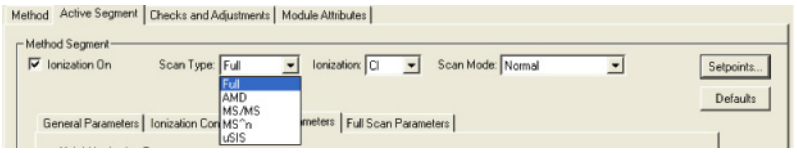

Adding or inserting a segment copies all of the parameters from the previous segment to the newly created segment. Double-click a field to edit the Segment Description, Start Time, or End time of a segment.

## Edit the method segments

This section describes editing parameters for Hybrid CI methods. For more information on performing Hybrid CI, see the section Building GC/MS Methods - Hybrid PCI and NCI in the 4000 GC/MS Software Operation Manual.

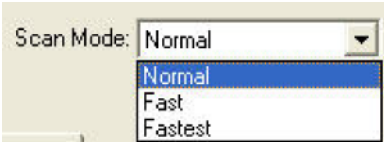
### Scan function settings

Select a Scan Type from the menu. In Hybrid configuration, CI is the only choice in the Ionization menu.




The 240 MS has three scan modes. The default scan mode is normal.

- **Normal**: This Scan Mode uses a prescan in Automatic Gain Control mode to determine optimum ionization time, and then ions are scanned at 5000 u/sec to collect the mass spectrum.
- **Fast:** This Scan Mode also uses a prescan in Automatic Gain Control mode to determine optimum ionization time, but ions are scanned at 10000 u/sec to collect the mass spectrum.
- **Fastest:** This Scan Mode uses ***no prescan*** and ions are scanned at 10000 u/sec to collect the mass spectrum. This mode is only available in Full scan type.



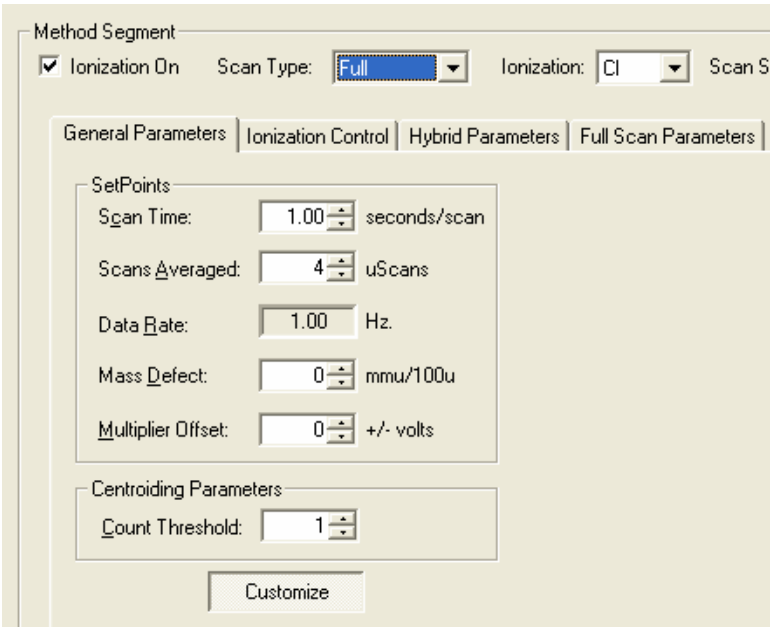

### General parameters tab

Scan Time, Scans Averaged, and Data Rate are linked. The number of scans averaged is updated when the scan time is adjusted and vice versa. To set the scan time, set the mass range and then change the scans averaged to three. The average of three scans gives the best compromise between a high chromatographic data rate and good spectral averaging.

Mass Defect allows for a systematic correction of the difference between the nominal mass of an atom (or ion) and its exact mass. Its importance arises from the fact that the NIST library reports molecular weights to the nearest integer mass unit only. The MS Workstation software must decide to which mass to assign measured intensity. If the exact mass of an ion happens to fall close to the dividing line between integer masses, the software may make an incorrect mass assignment. This scenario is more likely for molecules with higher molecular weights, since the mass defects for several atoms may add together to produce a sizable mass defect. For example, the exact mass for the lightest isotope form of $C_2Br_6$ is 497.51002, which could easily be assigned as either 497 or 498.

Multiplier Offset adjusts the EM voltage by as much as 300V relative to the current multiplier setting in the Module Attributes tab dialog in Manual Control (this is usually the $10^5$ gain value from Auto Tune). Sometimes better sensitivity is achieved, particularly in techniques such as MS/MS, when the multiplier voltage is increased. Note that this adjustment can be made on a segment-by-segment basis.

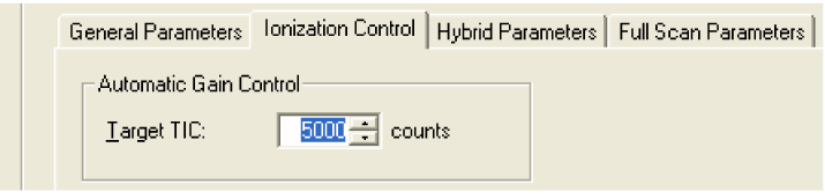
Count Threshold is normally 1; a value of 2 or 3 counts will reduce the number of low-level ions reported in the mass spectrum. This approach may improve library searches and reduce data file size at the cost of somewhat less detailed information in the mass spectra. The count threshold is shown only if the Customize button is active.




### Ionization control

Specify the Target Total Ion Current, or TIC. The Automatic Gain Control (AGC) algorithm uses the ion count from a prescan at fixed ion time, along with this target value, to calculate an ion time necessary to fill the ion trap with the target number of ions during the analytical scan. The objective is to fill the trap with an optimal number of ions during each analytical scan. The Target TIC is usually not set below 10,000 for full scan acquisitions, but it should also not be set too high or spectral distortions due to space charge may result (loss of MS resolution and/or shift in mass assignments for strong chromatographic peaks). Typically, a Target TIC between 20,000 and 40,000 counts gives the best results.

The default Target TIC for positive or negative chemical ionization is 5,000. The target can be set as high as 65,000. Click **Customize** to run fixed ion time experiments with ion times as high as 65,000 µsec or to change the Maximum Ion Time for CI Auto experiments. You can turn on the CI Gas and the ion trap in Manual Control and check the ion time in CI Auto mode.




### Hybrid parameters

The **Reagent Low Mass** and **Reagent High Mass** values are set to bracket the CI reagent ion mass range of interest. The Reagent Low Mass must be set to at least 10u below the mass of the lowest reagent ion of interest without a loss of intensity for that reagent ion. It is helpful to adjust these parameters in Manual Control with the **View** box checked in the field to the right of this dialog. The Reagent Low Mass parameter sets the RF storage level to exclude ions below the selected m/z. This is not a precise way to perform isolation. By contrast, the Reagent High Mass isolation step occurs after the ionization time, when resonant waveforms are applied to the ion trap end caps to eliminate ions with m/z above the selected Reagent High Mass.

**Ejection Amplitude** is the voltage of the waveforms for high mass isolation of CI reagent ions. The default value is 15V.

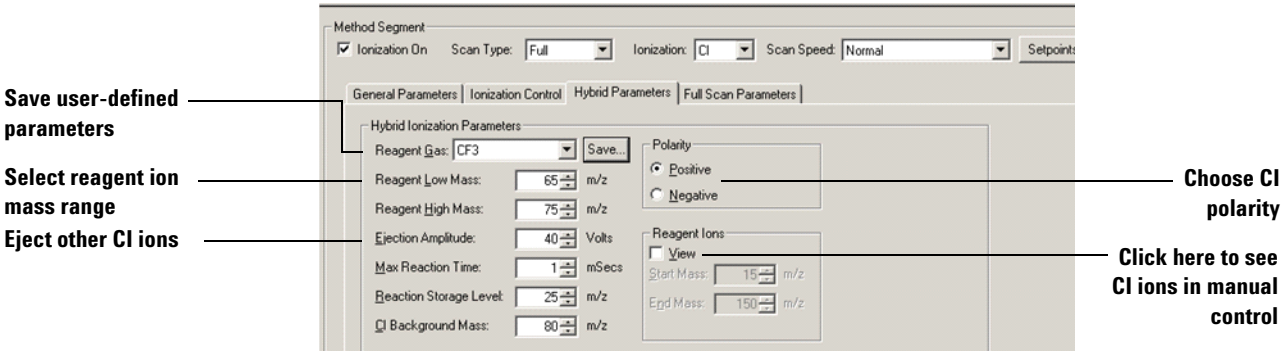
**Max Reaction Time** is the maximum time in sec allowed for CI reaction. If the ion time is reduced below the maximum based on the results of the prescan, the ion time will be scaled back proportionately. The allowed range for this parameter is 1 to 2000 µsec.

The **Reaction Storage Level** is the RF storage level in the ion trap during CI reaction, following the ionization period. It should not be set above the m/z of the CI reagent ion with which you wish to perform the CI reaction, else these ions will be ejected from the ion trap.

The **CI Background Mass** is the lowest m/z counted during the CI prescan. It can be higher than the low mass of the acquisition range but is usually set to be at or below the Start Mass value.

**Polarity** is selected for either positive or negative hybrid CI.

Set the **Start Mass** and **End Mass** ions to **View** in this dialog. Click the **View** box when the method is opened in Manual Control to observe the effects of Reagent Start Mass and Reagent End Mass isolation adjustments. Remember you must manually turn on the ion trap and CI gas icons to observe the CI reagent ions in this way.

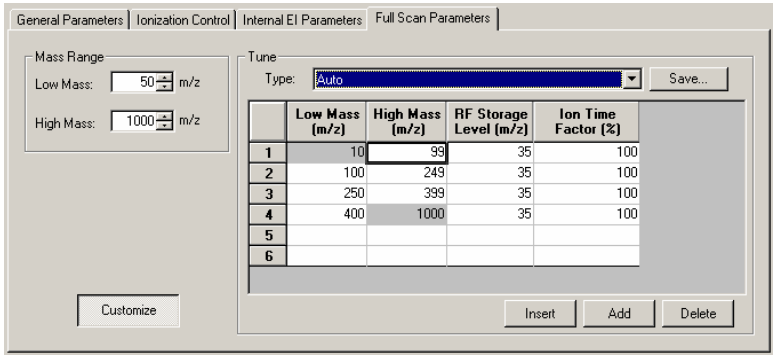



## Scan parameters

Each MS scan types has different parameters that need to be specified. Below are examples of the two most common scan types used in the Hybrid configuration, Full Scan and MS/MS. For detailed information on all scan types, see the section Building GC/MS Methods in the 240 GC/MS Software Operation Manual.

## Setting full scan parameters

To use only a single mass range segment in CI, type in the desired values for Low Mass and High Mass of the acquisition range. However, as the following shows, you can enter up to six non-contiguous mass ranges (separated by at least 10u). This feature can also be time programmed on a chromatographic segment basis. It is then possible to tailor the CI acquisition ranges for CI to different target analytes depending on the mass spectrum of each compound. An example of a four range acquisition is shown here:



## Setting MS/MS parameters

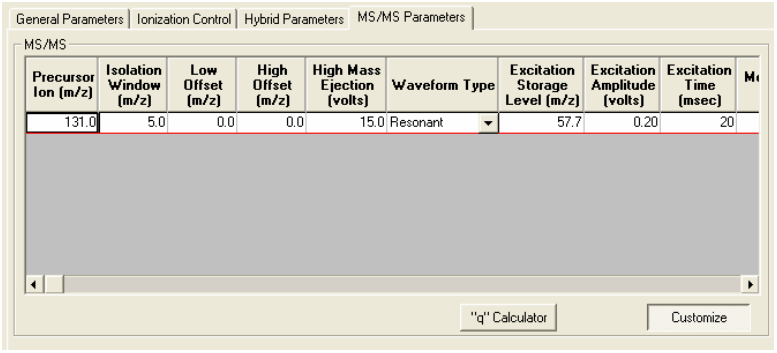
Tandem mass spectrometry, or MS/MS, uses ion preparation steps after the analyte ionization step and before mass analysis. MS/MS may be performed after either electron or chemical ionization. Briefly, all ions are eliminated from the stored mass range except at the m/z of a precursor ion. The precursor ions are then excited by waveforms applied to the ion trap. When enough energy is deposited in this way, collisions of precursor ions with helium buffer gas cause dissociation of the precursor ions to lower mass product ions. The remaining ions are then scanned to collect an MS/MS spectrum.

When well designed, an MS/MS method will:

- Fill the ion trap with only the selected precursor ions, so that trap capacity is used so that in many cases, co-eluting interfering compounds are excluded from the trap.
- Create product ions via a unique dissociation pathway, eliminating chemical noise.

MS/MS is useful only when the target compounds of an analysis are known. It is not useful for general qualitative analysis except to the degree one is determining a set of isomers of a given class, such as PCBs or Dioxins.

The next figure shows the MS/MS Parameters Tab Dialog.




**Precursor Ion (m/z)**: The precursor ion is the desired ion m/z that will be isolated in the MS/MS isolation step. This Precursor Ion m/z value is used in both Resonant and Non-Resonant Methods of MS/MS.

**Isolation Window (m/z)**: The full mass isolation window range is 1.0 to 14.0 m/z. The actual range is dependent on the precursor ion. The default value is 3.0 m/z. Integral and fractional mass

isolation window values are both accepted. If an isolation window smaller than 1.5 m/z is used, then the exact mass of the precursor ion should be entered in the Precursor Ion Mass field.

If the Low or High Edge Offset range is not sufficient to completely isolate the desired ions, either increase (in the case of desired ions not being present) or decrease (in the case of unwanted ions being isolated) the Isolation Window value.

**Low Edge Offset**: The mass offset to optimize the ejection of the mass just below the precursor ion mass. The Low Edge Offset range is –0.5 m/z to 0.5 m/z. The default value is 0.

Low Edge Offset affects the isolation window on the low mass side of the precursor ion. Increasing the mass offset (increasing the default from 0 to 0.1 m/z) makes the isolation window on the low mass side of the precursor ion larger. Decreasing the offset (decreasing from the default 0 to –0.5 m/z) decreases the window on the low mass side. The offset should be adjusted to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments.

**High Edge Offset**: The mass offset to optimize the ejection of the mass just above the precursor ion mass. The High Edge Offset range is –0.5 m/z to 0.5 m/z. The default value is 0.

High Edge Offset affects the isolation window on the high mass side of the precursor ion. Increasing the mass offset (increasing the default from 0 to 0.1 m/z) makes the isolation window on the high mass side of the precursor ion larger. Decreasing the offset (decreasing from the default 0 to -0.1 m/z) decreases the window on the high mass side. The offset should be adjusted to minimize the amplitude of the adjacent masses below the precursor ion. Initially, adjust in 0.2 m/z increments.

If the Low or High Edge Offset range is not sufficient to completely isolate the desired ions, either increase (in the case of desired ions not being present) or decrease (in the case of unwanted ions being isolated) the isolation window.

**High Mass Ejection**: Amplitude of broadband waveform used to eject masses above the isolated precursor ion. Default is 35 volts. If precursor ions are lost due to dissociation, reducing this amplitude may help. However, some ions with an m/z higher than the precursor ion may not be ejected.

**Waveform Type**: The waveform type is either resonant or non-resonant. Resonant waveforms are harmonious with the frequencies of electrons held in the ion trap. Non-resonant waveforms are not harmonious with the frequencies of the electrons held in the ion trap.

**Excitation Storage Level (m/z)**: The RF storage level in m/z when the dissociation waveform is applied following isolation. The excitation storage level range depends on the precursor mass, but the lowest product ion must be more than several mass units above the excitation storage level. A starting excitation storage level for a precursor ion can be calculated using the “q” calculator. The “q” calculator is accessed by right-clicking on any of the fields in the MS/MS parameters table.

The optimum excitation storage level is a tradeoff between a storage level high enough to allow fragmentation of the precursor ion and a storage level low enough to allow efficient trapping of the lowest m/z product ion. A higher excitation storage level allows more energy to be imparted to the precursor ions by using a higher excitation amplitude.

**Excitation Amplitude (volts)**: Voltage used to excite the precursor ion causing it to dissociate into product ions. The amplitude range for non-resonant excitation is 0 to 120 volts. For resonant excitation, the range is 0 to 60 volts. The default values are 0.2 volts for the resonant excitation method and 20 volts for the non-resonant excitation method.

If the excitation amplitude used is too large, the precursor ion and product ion spectra will be absent because both ions will be ejected from the trap. If the value is too small, the precursor ion spectrum will be dominant and the product ion spectrum will be weak or missing.

**Excitation Time**: The excitation time is the time required for collision-induced dissociation (CID) by ion excitation. The excitation time range is 0 to 650 msec. The default excitation time is 20 msec for both resonant and non-resonant excitation.

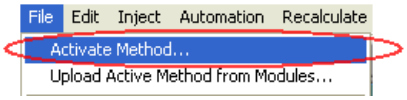
## Viewing method in manual control

After creating a method in the Method Builder, you can preview it in Manual Control. All MS parameters can be edited and previewed before a run. However, you cannot change the number of segments, or the start and end times of existing segments, unless you click Edit Method and see the Method Builder to make those changes.

### Activating a method

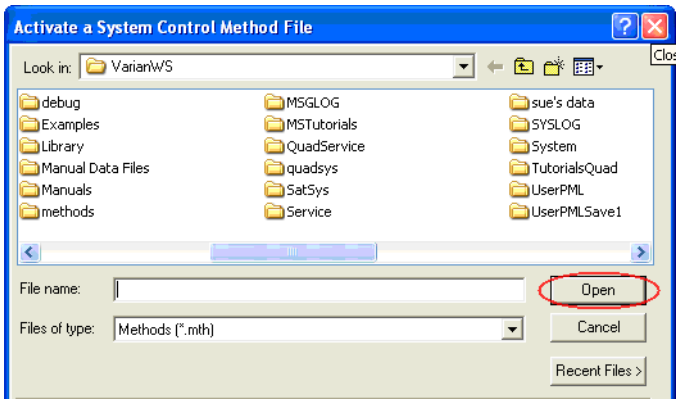
1 Click the **File** menu.

2 Click **Activate Method**.

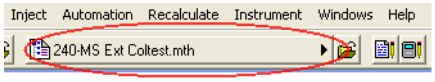



3 Select a method by either

- Clicking **Recent Files** to display the eight most recent methods.
- Clicking **Open** after selecting a method from a folder.

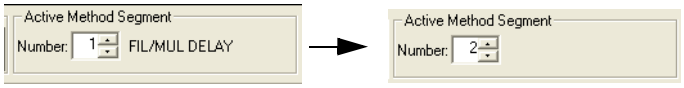


4 The active method is displayed in the toolbar.

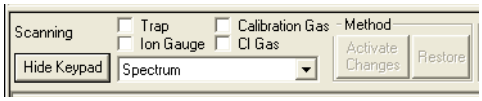


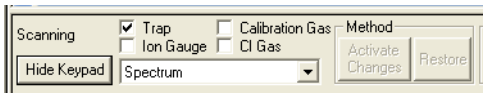

## Displaying ions

1 Select an ionization segment in which the ionization is on. You cannot turn on the ion trap in a segment where ionization is OFF as in the Fil/Mul Delay segment #1. Change to an ionization segment:

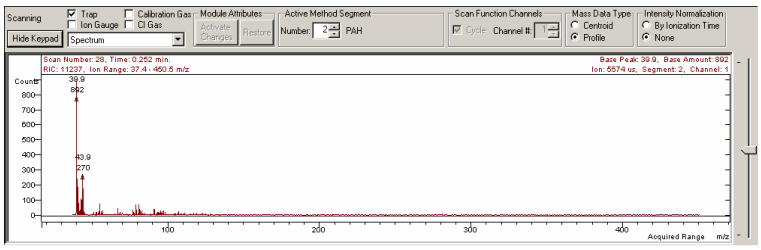



2 Click the Trap check box to turn on the ion trap.



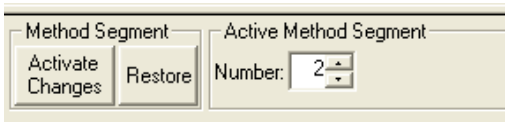

3 Select the method segment to view. Turn on the calibration gas or CI gas by selecting the check box.



### Editing a method in manual control

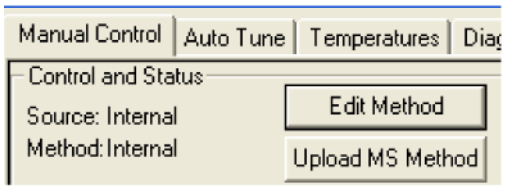
Examine and edit all the parameters in the active MS method and observe the changes on the mass spectra being acquired. The exact set of tab dialogs depends on the ionization and ion preparation modes in the current method segment.

After editing a parameter, implement the change by clicking the **Activate Changes** button as shown in the next figure.

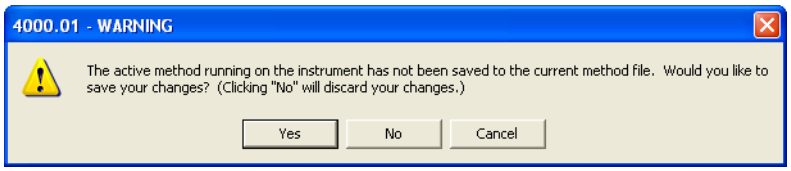



### Saving a method

1. Click the **Upload MS Method** button above the Ion Trap icon.
2. Click the **Edit Method** button, open the Method Builder, and make and save the changes.




If you do not upload changes, the method is checked to see if changes are made when the segment is changed or when you leave **Manual Control** or the **Method Segment**. If changes were made, you have the option to save or discard these changes.



# 4 Mode Conversion

For more detailed information on either of the following topics, see the 240 Hardware Operation Manual.

## Internal to Hybrid

Converting the 240 MS from Internal to Hybrid configuration involves changing only the ion source. The Internal ion source assembly is removed from the trap assembly and replaced with the External ion source assembly.

The transfer line orientation remains in the internal position.

1. Remove the analyzer assembly from the MS manifold.
2. Change the ion source to external.
3. Move the heat shield to the forward position.
4. Remove the filament adaptor and connect the flex cable.
5. Replace the analyzer in the MS manifold.

## External to Hybrid

Changing from External configuration to Hybrid configuration does not require changing the ion source assembly. However, the transfer line must be moved from front to rear position, and the transfer line tip must be changed to the internal type.

1. Change the transfer line entry location from external to internal.
2. Replace the external line tip with the internal tip.
3. Cut the column 1 mm past the transfer line tip.
4. Insert the Hybrid source plug.

## Effects of hardware changes

After changing the configuration, for example from External to Internal, the following occurs when you restart System Control:

- System Control compares the current configuration stored in the current Module Attributes with the configuration reported by the hardware.
- If these do not match, the Module Attributes are updated to the appropriate configuration. A similar process occurs for the default method (Default.mth).
- After making the hardware configuration change, new methods will have the appropriate instrument configuration by default.

The resetting of Module Attributes requires you to run all Auto Tune routines, since the prior Auto Tune results are invalid.